

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
SPIRODICLOFEN (BAJ 2740)

Chemical Code # 5857, Document Processing Number (DPN) # 52944
SB 950 # N/A
6/9/06

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, no adverse effect
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, possible adverse effect
Reproduction, rat:	No data gap, possible adverse effect.
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	No data gap, no adverse effect

Toxicology one-liners are attached.

All record numbers for the above study types through 221586 were examined.

In the 1-liners below:

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study on file but not yet reviewed.

File name: T060509A

Original: T. Moore, 5/9/06; Revised: P. Leung, 6/9/06

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

NOTE: A stability study for spirodiclofen in rodent ration is found in DPR Document No. 52944-0047, Record No. 218896 (one of 3 short reports under this Record Number). Bayer Report No. 108164, Moore, K. D. and C. J. Brenneke, "The homogeneity and stability of BAJ 2740 in rodent ration," 11/13/98. Page 12 of this record indicates that stability of spirodiclofen in rodent diet at 10000 ppm is much higher than at 25 ppm. At 10000 ppm, 78.5% of the initial test article content was present at day 14, whereas decline in 25 ppm content (as percent of initial content) was as follows: day 1 (83%), day 3 (71%), day 7 (55%), day 10 (49%), and day 14 (41%). Additional stability studies associated with individual animal studies confirm the limited stability of low dietary concentrations of spirodiclofen. (Aldous, 12/27/05. No worksheet.)

COMBINED, RAT

****52944-0037 218881** Wirnitzer, U., U. Bach, and E. Hartmann, "BAJ 2740: Combined study on chronic toxicity and carcinogenicity in Wistar rats (Dietary administration over 2 years)," Bayer AG, Department of Toxicology, Wuppertal, 10/27/2000. Bayer AG Study No. T7061640. Sixty Wistar rats (Hsd Cpb:WU) per sex per group were administered Spirodiclofen (BAJ 2740) in diet in a combined chronic/oncogenicity study: 50/sex/group designated for lifetime (108 wk) exposure, and 10/sex/group for 1-yr interim sacrifice. Mean purity of the six batches utilized was 98.0%. Design included an FOB on 10 rats/sex/group at week 77. Dose levels were 0, 50, 100, 350, and 2500 ppm, achieving exposures of 2.0, 4.1, 14.7, and 110 mg/kg/day in M, and 0, 2.9, 5.9, 19.9, and 153 mg/kg/day in F. NOEL = 350 ppm [based primarily on body weight decrements of about 7 to 8% in both sexes, testicular interstitial cell adenomas and hyperplastic foci, uterine adenocarcinomas, vacuolated enterocytes in jejunum, adrenal cortical diffuse hypertrophy and vacuolation (M only), consistently elevated alkaline phosphatase, and generally reduced cholesterol and triglyceride levels]. Small but statistically significant increases over controls for the following common findings, in males only, were justifiably considered by investigators as treatment-related: atrophy and/or degeneration of nasal olfactory epithelium and colloidal alteration of the thyroid. Study is acceptable, with the uterine and testicular tumors as "possible adverse effects." Aldous, 3/17/06.

52944-0038 218882 is a supplement to the combined study (52944-0037 218881), and consists of historical incidence or mean value data on hematology, clinical chemistry, urinalysis, and pathology.

CHRONIC TOXICITY, RAT

See "Combined, Rat," above

CHRONIC TOXICITY, DOG

****52944-0039, 0036 218883 and 218880**, "BAJ 2740, Chronic Toxicity Study in Beagle Dogs (One Year Feeding Study)", (H. Wetzig and C. Rühl-Fehlert, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30829, Bayer AG Study No. T 6 067 346, Bayer Inc., Report No. 110517, 9 February 2001). 4 Beagle dogs per sex per group received BAJ 2740 (97.8% spirodiclofen) in the diet (moistened 1:1 with water) at 0 (standard diet), 20, 50, 150, and 500/600 ppm for 52 weeks. The dietary concentration for the 500 ppm group was increased to 600 ppm in week 4 (day 22). Mean BAJ 2740 intake was 0.56, 1.38, 4.33, and 16.1 mg/kg/day for males and 0.59, 1.52, 4.74, and 17.7 mg/kg/day for females at 20, 50, 150, and 600 ppm respectively during the 52-week treatment period. No treatment-related changes in clinical signs, behavior, reflexes, body temperature, blood pressure, mortality, bodyweight, food consumption, urinalysis, and hematology results were recorded. In liver tissue at necropsy, dose-dependent increases in N-demethylase activities were noted from 50 ppm

and higher. O-demethylase was slightly increased at 600 ppm. Group mean relative and absolute adrenal weights were increased in both sexes at the high dose level. In males, relative testes weights were increased at 150 and 600 ppm along with relative prostate weights at the high dose level (values for both were within the historical control range). In testes, histopathology revealed Leydig cell hypertrophy and vacuolation and bilateral focal tubular degeneration at the high dose level. In adrenal glands, dose-dependent increases (incidence and grade) of cortical vacuolation in the zona fasciculata were noted at 150 ppm and higher in both sexes. Chronic NOEL = 50 ppm (M: 1.38 mg/kg, F: 1.52 mg/kg)(based on adrenal cortical vacuolation). Possible adverse effect: vacuolation of the adrenal cortex. Record 218880 contains historical control data for clinical pathology in dogs. Acceptable. (Green and Leung, 4/6/06).

ONCOGENICITY, RAT

See "Combined, Rat, above.

ONCOGENICITY, MOUSE

****52944-0040 218884** Wahle, B. S., "Technical grade BAJ 2740: An oncogenicity testing study in the mouse, Bayer Corporation, Stilwell, KS, 7/21/00. Bayer Corp. Agric. Div. Report No. 109626. Fifty CD-1[ICR]/BR mice/sex/group were dosed in diet at 0, 25, 3500, and 7000 ppm Spiroclufen [(BAJ 2740), purity 98.6%] for 18 months, achieving respective mean intakes of 4.1, 610, and 1216 mg/kg/day in treated males and 5.1, 722, and 1495 mg/kg/day in females. NOEL = 25 ppm. Both sexes at 3500 and 7000 ppm had elevated hepatocellular adenomas and/or carcinomas [incidences of 1, 1, 8*, and 10** in control through high dose males, respectively; and incidences of 0, 0, 5*, and 3 in corresponding females (*, ** = significant, $p < 0.05$ and $p < 0.01$, respectively)]. Both sexes at 3500 and 7000 ppm had vacuolization of the adrenal cortex. There was markedly increased pigmentation of the cortico-medullary junction of the adrenals in 3500 to 7000 ppm females and in 7000 ppm males. Males had greatly increased incidence of testicular interstitial cell hypertrophy/hyperplasia at 3500 to 7000 ppm. Hepatocytomegaly was sharply elevated in males at 3500 to 7000 ppm, with no corresponding effect in females. Increased incidence and/or degree of amyloid formation was found in several organs at the top two dose levels, and appears to be treatment-related in that range in both sexes. Typically, absolute organ weights were elevated in both sexes at 3500 and 7000 ppm for adrenals and liver, also in testes of males. Kidney absolute weights were significantly decreased at these dose levels in both sexes. Incidence of testicular degeneration was elevated at 7000 ppm, as was incidence of epididymal aspermia. Incidence of atrial thrombus was significantly elevated in 7000 ppm males. Study is acceptable, with deficiencies noted. Liver tumors are "**possible adverse effects.**" Aldous, 3/23/06.

52944-0040 218884 (continuation: the 6-week dietary range-finding study for above Record No. 218884 is found on pp. 2264 ff. of that record). This pilot study employed dietary dose levels of 0, 25, 50, 500, 2500, 5000, and 7000 ppm Spiroclufen [(BAJ 2740), purity 98.2%] in 10 CD-1 mice/sex/group. Body weight, and food consumption did not demonstrate treatment effects. Clinical signs were not considered by investigators to be treatment-related, however "rough coat" (commonly associated with associated perigenital urine staining) was observed 4 times in high dose males, vs. 0 to 2 times in other groups of males, with no indication of dose-response in lower dose groups (Table 3 of range-finding report). This reviewer considers these findings to indicate a treatment response. Clinical chemistry was generally uneventful except that 5000 and 7000 ppm females each had reduced cholesterol compared to other groups (mean levels of 87, 81, 94, 89, 74, 57*, and 58* mg/dl, the latter two significant ($p < 0.05$) compared to concurrent controls). There were only 5 mice/sex/group evaluated for clinical chemistry and only one sampling period evaluated, hence data should be interpreted with caution. Hematology was performed on as few as one/group in most male groups, and is of no interpretative value. Hematology in females was uneventful. Absolute liver weights were elevated in 5000 and 7000 ppm rats (mean male liver weights of 1.9, 2.0, 2.1, 2.2, 2.1, 2.3*, and 2.6* g in controls through high dose groups, respectively, and in females: 1.5, 1.5, 1.7, 1.6, 1.7,

1.9*, and 1.8* g, respectively). [Data from Table 6 of range-finding report: * = significant, $p < 0.05$]. The brief report of the 6-wk study did not tabulate histopathology data, however the text (p. 2266) stated the following: "Compound-related changes were identified histopathologically and included centrilobular hepatocytomegaly (7000 ppm M and F), and vacuolization of the adrenal cortices (5000-7000 ppm M and 500 to 7000 ppm F). Gross pathological evidence of toxicity was not observed in this study." Thus the highest two values in the definitive study were well-chosen with respect to the range-finding study. Aldous, 3/23/06 (no worksheet).

REPRODUCTION, RAT

****52944-0035 218878** Eiben, R., "BAJ 2740: Two-generation study in Wistar rats" (Revised report with amendment attached). Bayer AG, Department of Toxicology, Wuppertal, 2/15/00. Bayer AG Study No. T0061977. Groups of 25 Wistar [CrI:WI(WU) BR] rats/sex/group were dosed in diet with Spirodiclofen (BAJ 2740), purity 98.6, during pre-mating [82 days (F0) and 89 days (F1)], and continuously through one mating period per generation, and through respective lactation periods (28 days) in a standard reproduction study. Levels of 0, 70, 350, and 1750 ppm provided pre-mating mean F0 doses of 5.2, 26, and 135 mg/kg/day for M and 5.5, 28, and 139 mg/kg/day for F. Associated pre-mating mean F1 doses were 6.4, 30, and 178 mg/kg/day for M and 7.0, 34, and 193 mg/kg/day for F. Investigators performed histopathology of reproductive and potential target organs on all control and high dose parental F0 and F1 rats, plus several potential target organs on intermediate group adults. Parental systemic toxicity NOEL = 70 ppm (modest body weight decrements by the middle of lactation in females and at comparable age in males of both generations; modest vacuolation of the cytoplasm of the adrenal cortical zona fasciculata, which was statistically significant in F0 350 ppm females and non-significantly elevated, but plausibly treatment-related, in F1 350 ppm females). Parental reproductive effects NOEL = 350 ppm (4/25 F1 high dose males were infertile, associated with testicular atrophy, epididymal atrophy and oligospermia). The four infertile males had zero spermatid head counts in testes, and zero spermatazoa counts in epididymides, whereas there were no differences in these counts in the remaining high dose males nor in 350 ppm males compared to controls. Sperm morphology and motility were unaffected by treatment at any dose tested. The 4 infertile males had exceptionally low body weights, suggesting that reproductive toxicity may have been a non-specific reflection of general toxicity at a dose exceeding the MTD based on body weight decrements. Offspring viability and growth NOEL = 70 ppm (very small but statistically significant mean pup weight decrements in F1 pups at day 21). Common findings in adults at 1750 ppm included body weight decrements (7% and 15% for F0 M and F rats at Week 19, and 17% and 10% for F1 M and F rats at Week 19); elevated relative adrenal gland weights in F0 and F1 males and females; clinical chemistry changes in F1 rats of sharply elevated alkaline phosphatase and reduced cholesterol, triglycerides, and free fatty acids; and epithelial vacuolation of the epithelial cells of the jejunal villi in high dose F0 males. An important finding in 1750 ppm group offspring of F1 rats was increased post-implantation (pre-natal) mortality. Several observed findings in the 1750 ppm group are **"possible adverse effects,"** particularly male infertility (with associated histopathology of reproductive structures), marked pup body weight decrements during lactation and beyond, and post-implantation losses. This dose level should be considered as "excessive" with respect to parental toxicity, showing major body weight decrements and histopathology in multiple organs: typically with sharp dose-response curves. Acceptable. Aldous, 3/21/06.

52944-0047 218897 Eiben, R., "BAJ 2740: one-generation study in Wistar rats," (pilot study for the primary reproduction study). (Bayer AG Report No. 26960, Bayer Corp. Agric. Division Report No. 108545, 12/15/97). Ten rats/sex/group were dosed in diet for 4 weeks prior to mating and through until necropsy with 0, 250, 2500, or 10000 ppm spirodiclofen (98.6% purity). Estimated pre-mating spirodiclofen intakes were 14, 139, and 536 mg/kg/day in treated males, and 18, 172, and 732 mg/kg/day in females. Litters were culled at day 4, then carried for 4 weeks after birth. Parental rats and F1 pups were then grossly examined, without

histopathology exams. Body weights were remarkably reduced in 10000 ppm parental rats (16% in M, 17% in F) and in 2500 ppm females (10% decrement). Kidney and liver absolute weights were proportionally reduced in both sexes at 10000 ppm, as were kidney weights in 2500 ppm females. In addition, ovarian weights were significantly reduced at 2500 and 10000 ppm. There were no definitive effects of treatment on pregnancy outcomes. Pup weights were statistically significantly reduced compared to concurrent controls in all treatment groups during lactation, although 250 ppm pup weights were only marginally below control weights of the primary study (compare p. 38 of pilot vs. pp. 147-148 of primary study). At gestation day 28, substantial pup body weight decrements were found at 2500 and 10000 ppm: 2%, 25%, and 66% reductions in 250, 2500, and 10000 ppm male weanlings, respectively, and 5% (marginally statistically significant, probably incidental), 24%, and 61% reductions in females. Pup survival was substantially reduced at 10000 ppm prior to culling and on through weaning. There were no characteristic gross changes in the pups. Two 10000 ppm females had "thickened" mammary glands, compared to none in other groups. There were no other gross findings in parental rats. Based on this study, dose levels chosen for the primary study were justified. Aldous (examined in conjunction with the primary study), 3/21/06.

TERATOLOGY, RAT

**52944-0034 218877, "BAJ 2740, Developmental Toxicity Study in Rats After Oral Administration", (A. M. Klaus, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 29736, Bayer AG Study No. T2061366, Bayer Inc. Report No. 110545, 28 March 2000). 28 mated female Wistar Hsd Cpb:WU rats per group received BAJ 2740 (97.9% spirodiclofen) by oral gavage at 0 (0.5% carboxymethylcellulose in demineralized water), 100, 300, and 1000 mg/kg/day on gestation days 6 through 19. There were no treatment-related changes to maternal clinical signs, mortality, food and water consumption, excreta, bodyweight and bodyweight change, reproduction parameters, or necropsy findings. Fetal numbers, weight, morphology, sex distribution, visceral, and skeletal findings were not affected by treatment. Maternal and developmental NOEL = 1000 mg/kg/day. No teratogenicity. Acceptable. (Green and Leung, 4/6/06).

TERATOLOGY, RABBIT

**52944-0033 218876, "BAJ 2740, Developmental Toxicity Study in Rabbits After Oral Administration", (B. Holzum, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. 27631, Bayer AG Study No. T1061725, Bayer Inc. Report No. 108874, 1 July 1998). 22 mated female Himalayan CHBB:HM rabbits per group received BAJ 2740 (97.9% to 98.5% purity, mixed batch No. 06480/0002) by oral gavage at 0 (0.5% carboxymethylcellulose), 100, 300, and 1000 mg/kg/day on gestation days 6 through 28. No treatment-related maternal deaths were indicated (one death at 300 mg/kg/day was due to intestinal blockage). Maternal food consumption was significantly reduced at 300 (gestation days 6-9) and 1000 mg/kg/day (days 6-15) and bodyweight gain was significantly reduced for gestation days 6-9 at 300 and 1000 mg/kg/day. At 1000 mg/kg/day, female No. 3318 had nearly no food intake and severe bodyweight loss (452 g) from gestation day 6 until abortion (day 20). An increase in the incidence of reduced feces was noted at 300 and 1000 mg/kg/day and 14 dams at 1000 mg/kg/day had a light discoloration of the feces. Decreased water consumption and urination and increased alopecia were also recorded at 1000 mg/kg/day. Necropsy results for the dams were generally unremarkable. High dose female (No. 3318), which aborted on gestation day 20, showed distinct liver lobulation (one control female also showed liver lobulation on day 29). The number of litters, mean litter size, and mean fetal weight were comparable across groups. At 1000 mg/kg/day, 14 fetuses from 3 litters were noted with distinct liver lobulation (deviation) compared to 3 control fetuses from 2 litters. The fetal incidence (10.1%) was statistically significant and greater than the range of historical control incidence for this strain of rabbit. Treatment-related fetal skeletal changes were not indicated. No teratogenicity. Maternal NOEL = 100 mg/kg/day (decreased food consumption, decreased bodyweight gain). Developmental

NOEL = 300 mg/kg/day (liver lobulation). Acceptable. (Green and Leung, 4/6/06).

GENE MUTATION

****52944-0041** 218888, "BAJ 2740, *Salmonella*/Microsome Test, Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. 25325, Bayer AG Study No. T 3053744, Bayer Inc., Report No. 107789, 8 August 1996). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to BAJ 2740 (99.1% spirodiclofen), in the presence and absence of S9, at 0 (DMSO), 16, 50, 158, 500, 1581, and 5000 µg/plate for 48 hours at 37°C. Two trials were performed. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated for 20 minutes (37°C) prior to plating. There was no increase in the number of revertants per plate and no indication of bacterial toxicity (reduced titer) at any treatment level. Positive controls were functional. Acceptable. (Green and Leung, 4/12/06).

52944-0042 220243, "BAJ 2740, Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HPRT Assay in Vitro", (S. Brendler-Schwaab, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. 25974, Bayer AG Study No. T 8053749, Bayer Inc., Report No. 107784, 10 February 1997). Exponentially growing V79 cells (4×10^6) were plated (250 ml flasks) in duplicate and treated with BAJ 2740 (99.1% spirodiclofen) for 5 hours at untreated, 0 (DMSO), 4, 6, 8, 10, 15, and 20 µg/ml in the absence of rat liver S9 and at untreated, 0, 10, 20, 40, 50, 60, and 80 µg/ml in the presence of S9. No increase in forward mutations was indicated. Unacceptable and possibly upgradeable with submission of complete results for first non-activated trial. (Green and Leung, 4/12/06).

52944-0041 220247, "BAJ 2740 240 SC, *Salmonella*/Microsome Test Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30756, Bayer AG Study No. T 5069541, Bayer, Inc., Report No. 110529, 19 February 2001). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to BAJ 2740 240 SC (243.0 g BAJ 2740/l), in the presence and absence of S9, at 0 (deionized water), 16, 50, 158, 500, 1581, and 5000 µg/plate for 48 hours at 37°C. Two trials were performed. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated for 20 minutes (37°C) prior to plating. There were no increases in the numbers of revertants per plate at any level. Positive controls were functional. Strain specific bacterial toxicity (reduced titer) was recorded at 1581 and 5000 µg/plate in Trial 1 and at 5000 µg/plate in Trial 2. Supplemental to BAJ 2740 data. (Green and Leung, 4/12/06).

CHROMOSOME EFFECTS

****52944-0042** 218890, "BAJ 2740, *In Vitro* Mammalian Chromosome Aberration Test with Chinese Hamster V79 Cells", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH-25716, Bayer AG Study No. T7053748, Bayer Inc., No. 107785, 3 December 1996). 1×10^6 Chinese hamster V79 cells were seeded in duplicate in 20 ml of medium per 75 cm² flask and exposed (4 hours) to BAJ 2740 (99.1% spirodiclofen) in the absence of S9 at 0 (DMSO), 0 (untreated), 0.75, 1.5, 3, 5, 6, 10, 12, 20, 40, 80, and 160 µg/ml and, in the presence of S9, at 0 (DMSO), 0 (untreated), 10, 20, 40, 80, and 160 µg/ml. Cells were harvested and evaluated 18 and/or 30 hours after the start of treatment. 100 metaphases per culture were evaluated by light microscopy. Cytotoxicity (decreased mitotic index) was noted at 0.75 µg/ml and higher in the absence of S9 and at 80 µg/ml and above with activation. Aberrant metaphases were not increased by treatment with BAJ 2740. Positive controls were functional. Acceptable. (Green and Leung, 4/12/06).

52944-0042 220244, "BAJ 2740 240 SC, *In Vitro* Chromosome Aberration Test with Chinese

Hamster V79 Cells", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30826, Bayer AG Study No. T 4069883, Bayer Inc., No. 110534, 15 March 2001). 1×10^6 Chinese hamster V79 cells were seeded in duplicate in 20 ml of medium per 75 cm² flask and exposed (4 and 18 hours) to BAJ 2740 SC 240 (243 g BAJ 2740/l) in the absence of S9 at 0 (deionized water), 0.001, 0.005, 0.01, 0.02, 0.04, and 0.05, 0.1, and 0.5 µl/ml and, in the presence of S9 with 4 hour exposure, at 0, 0.001, 0.005, 0.01, and 0.05, and 0.1 µl/ml Cells were harvested and evaluated 18 and/or 30 hours after the start of treatment. 8, 18, and 30 hour harvest times were used for mitotic index determinations (4 hour treatment). Cells treated for 18 hours were evaluated for cytotoxic effects at 18 hours. An additional assay with 8 hour treatment (non-activated) and harvest times was performed solely for mitotic index determinations. Cytotoxic effects (decreased mitotic index) were noted at 0.05 µl/ml and higher after treatment for 4 hours in the presence and absence of S9. In the absence of S9, the mitotic index was reduced after treatment for 8 hours at 0.01 µl/ml and higher and at 0.02 µl/ml and higher after treatment for 18 hours. Under non-activated conditions, changes in cell morphology were reported at 0.5 µl/ml after 4 hours treatment and at 0.02 µl/ml and above after 18 hours. In the presence of S9, changes in cell morphology were reported at 0.05 µl/ml and higher. Precipitation in the medium was also reported at 0.01 µl/ml and higher. No increase in aberrant metaphases. Positive controls were functional. Supplemental to BAJ 2740. (Green and Leung, 4/12/06).

DNA DAMAGE

**52944-0042 220245, "BAJ 2740, Micronucleus Test on the Mouse", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH-25358, Bayer AG Study No. T 7060236, Bayer Inc., Report No. 107788, 20 August 1996). Groups of 5 Hsd/Win: NMRI mice per sex received BAJ 2740 (99.1% spirodiclofen) once by intraperitoneal injection at 0 (0.5% aqueous Cremophor emulsion) and 800 mg/kg followed by bone marrow sampling 16, 24, and 48 hours later. One animal died during the test period. Treated animals showed the following symptoms until sacrifice: apathy, roughened fur, spasm, and eyelids stuck together. Feeding behavior was normal. Slides of the bone marrow were evaluated by light microscopy. 1000 polychromatic erythrocytes were examined per animal. There was no increase in micronucleated polychromatic erythrocytes. Acceptable. (Green and Leung, 4/12/06).

NEUROTOXICITY

Rat Acute Neurotoxicity Study

0043; 218891; "An Acute Oral Neurotoxicity Screening Study with Technical Grade BAJ 2740 in Wistar Rats" (Sheets, L.P. and Gilmore, R.G., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Agricultural Division Report No. 109629, Study No. 98-412-TQ, 12/04/00). 818. Technical BAJ 2740 (Mixed Batch No. 06480/0002, purity = 97.7-97.9%) was suspended in 0.5% methylcellulose/0.4% Tween 80 in deionized water and administered by gavage in a single dose to 12 Wistar (CrI:WI(HAN)BR) rats per sex per dose at dose levels of 0 (vehicle only), 200, 500, and 2000 mg/kg. No mortalities occurred. No treatment-related clinical signs were observed during cageside observations. FOB observations and motor activity assessments revealed no treatment-related effects on the day of treatment (approximately 4 hours after treatment), and 7 and 14 days after treatment. Macroscopic and microscopic examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M/F) = 2000 mg/kg (based on no effects at the highest dose tested). **Acceptable.** (Corlett and Leung, 11/28/05)

Rat Subchronic Neurotoxicity Study

0044; 218892; "A Subchronic Neurotoxicity Screening Study with Technical Grade BAJ 2740 in Wistar Rats" (Sheets, L.P. and Gilmore, R.G., Bayer Corporation, Agriculture Division, Toxicology, Stilwell, KS, Agricultural Division Report No. 109808, Study No. 99-N72-AZ, 03/05/01). 827. Technical BAJ 2740 (Mixed Batch No. 06480/0002, purity = 97.4-97.8%) was

admixed to the feed (containing 1% corn oil by weight plus acetone) and fed to 12 Wistar ((CrI:WI(HAN)BR) rats per sex per dose at dose levels of 0 (diet, corn oil, and acetone only), 100, 1000, or 12500 ppm (0, 7.2, 70.3, and 1088.8 mg/kg/day, respectively for males and 0, 9.1, 87.3, and 1306.5 mg/kg/day, respectively for females) for 13 weeks. No mortalities occurred. Cageside observations and FOB observations revealed urine stained body in both males and females at 12500 ppm. A treatment-related decrease in mean body weight was observed in both sexes at 12500 ppm. Motor and locomotor activity assessments revealed no statistically significant effects. Necropsy revealed no treatment-related internal gross lesions. Neuropathological examinations revealed no treatment-related abnormalities. **No adverse effects.** NOEL (M)= 70.3 mg/kg/day (1000 ppm) and NOEL (F) = 87.3 mg/kg/day (1000 ppm) based on urine stained body and a decrease in body weight. **Acceptable.** (Corlett and Leung, 12/05/05)

Rat Developmental Neurotoxicity Study

****52944-0058 218928** Sheets, L. P. and S. G. Lake, "A developmental neurotoxicity screening study with technical grade spirodiclofen in Wistar rats," Bayer Corporation, Stillwell, KS, 6/30/04. Bayer Corp. Agric. Div. Report No. 201056. Thirty mated Wistar Hannover CrI:WI (GlX/BRL/Han) IGS BR dams per group (to obtain at least 20 suitable litters/group) were dosed with dietary spirodiclofen (purity 97.1%) from gestation day 0 through PND 21 in a standard developmental toxicity study at 0, 70, 350, or 1500 ppm (equivalent to 0, 6.5, 32, and 136 mg/kg/day during gestation, and 0, 14, 70, and 274 mg/kg/day during lactation). Maternal NOEL = 350 ppm (slight but statistically significant body weight and food consumption decrements during lactation). There was a statistically significant body weight decrement of about 8% during lactation at 1500 ppm on PND 21, with no treatment differences by PND 35. Pup NOEL = 350 ppm. Morphometric measurements of the parietal cortex width in males were statistically significantly reduced in 1500 ppm males at PND 75 sacrifice (1.88 mm for controls, 1.75 mm for 1500 ppm), without corresponding change in females. Intermediate groups were not measured for this parameter. Intermediate group data for parietal cortex width, if obtainable, might reduce the uncertainties in study interpretation. Historical control values may be useful. A validation study was provided to support this study (see review of 52944-0062 223561). A 4/3/06 revision of Record No. 218928 with revisions on pp. 5 and 55 was submitted. This information does not change conclusions of this review. Note that U.S. EPA has a different conclusion than DPR on interpretation of water maze data (see this review). **No adverse effects indicated. Acceptable,** however clarifying information is requested to clarify morphometric data results. Aldous, 5/3/06.

METABOLISM

****52944-0045 221358** Andersch, I. and J. Köster, "[Dihydrofuranone-3-¹⁴C]BAJ 2740: Investigation of biokinetic behaviour and the metabolism in the rat," Bayer AG, Leverkusen, FRG, Sept. 4, 2000. Bayer Corp. Agric. Div. Report No. 110646. Groups of Wistar Hsd/Cpb: Wu rats (about 200 g at treatment) were dosed with labeled spirodiclofen (radiopurity > 98%) in 10 ml/kg of 0.5% CMC suspension as follows (showing group designations in brackets): [6] single high dose (100 mg/kg, 4 M); [7] single low dose (incl. CO₂ measurement) (2 mg/kg, 4 M); [8] single low dose (EPA basic test) (2 mg/kg, 4 M); [9] single low dose (2 mg/kg, 4 F); [10] 14 daily doses with 2 mg/kg/day non-radioactive a.i., then 1 labeled low dose (2 mg/kg, 4 M); and [13] single low dose, bile cannulation study (2 mg/kg, 6 M). In low dose groups, about 70% of administered dose was absorbed, with most of the label found in the urine, and about 12% of administered dose found in the bile. In the high dose (100 mg/kg) group, 61% of label was found in feces, vs. 35% in urine, suggesting saturable absorption at high dose levels. Very little label (0.05% of administered dose) was found in exhaled CO₂. Peak plasma concentrations were observed between 2.5 hr to 3.9 hr in low dose groups, vs. 5.6 hr in high dose rats. Plasma radioactivity typically dropped about 10-fold in all groups between 8 hr and 24 hr after dosing (plasma phase 1 elimination t_{1/2} values were 2.4 hr to 4.2 hr). This is consistent with swift

clearance from organs and tissues as reported in Record No. 218893. There was a sex difference in urinary metabolites: low dose females excreted 53% of administered dose as the enol, whereas low dose males excreted low amounts of the enol (< 5%), but instead favored subsequent hydroxylation of the cyclohexyl moiety of the enol at carbon 3 or 4. Positions of the ring hydroxyls in the plane of the ring (designated "e" for equatorial) were most abundant: low dose males had 26% to 30% of administered label as the 3-hydroxy-enol (e) metabolite, and 13% to 15% of administered label as the 4-hydroxy-enol (e) metabolite. The associated axial "a" isomers with the hydroxyls perpendicular to the ring were comparatively minor metabolites. The combined 3- and 4-hydroxy-enol metabolites in females constituted only 17% of administered dose. There were no other common urinary metabolites. Pre-treatment with unlabeled low doses of spirodiclofen for 2 weeks had no obvious effect on metabolism. Fecal metabolism yielded 1 to 4% of parent spirodiclofen after low dose exposure, compared to 16% in high dose males (consistent with reduced absorption). The enol constituted 4 to 7% of administered dose in feces of low-dose non-cannulated rats (16% in high dose M), with 3- and 4-hydroxy-enol (e) metabolites as modest contributors (1% to 7% of administered dose for each of these isomers). Fecal metabolites included a few percent of mandelic acid-cyclohexyl-methyl esters (created by oxidatively opening the 5-membered ring at the location of the enol hydroxyl group), and subsequent metabolic products. Glucuronides were not observed in feces. The two most common bile residues were the OH-enol glucuronide and 3-hydroxy-enol (e) (3% and 4% of administered dose, respectively). This study adequately characterized the metabolic fate of spirodiclofen, and indicates no adverse effects. Aldous, 3/24/06.

52944-0045 218893 Köster, J., "[Dihydrofuranone-3-¹⁴C]BAJ 2740: distribution of the total radioactivity in the rat determined by quantitative whole body autoradiography," Bayer AG, Leverkusen, FRG, 7/17/00. Bayer Corp. Agric. Div. Report No. 109854. Five Wistar Hsd/Cpb: WU male rats were each dosed once by gavage with approximately 3 mg/kg Spirodiclofen (BAJ 2740), radiopurity > 98%, in 10 ml/kg of 0.5% CMC suspension. Rats were sacrificed at 1, 4, 8, 24, or 48 hr, with collection of tissues and urine. At sacrifice, rats were fixed in a stretched position using a template, and frozen. Slices were prepared at 50 µm thickness by longitudinal sections designated as median, paramedian, "adrenal gland section," and "eye-kidney section". Slices were freeze-dried, and developed on imaging plates. Label was quickly absorbed and distributed, with peak tissue levels between 4 and 8 hours, and very low to undetectable levels by 48 hr. About 59% of label was excreted in urine within 48 hr of dosing. Feces were collected, but not analyzed in this study. This supplementary study covers the tissue distribution component of the metabolism data required by guidelines. Aldous, 3/24/06.

52944-0051 218908 Schmidt, U., "BAJ 2740: Determination of BAJ 2740 and the enol of BAJ 2740 in plasma of rats in a chronic study. Bayer AG, Department of Toxicology, Wuppertal, 10/30/2000. Bayer Corp. Agric. Div. Report No. 110538. Both BAJ 2740 (spirodiclofen) and its enol (i.e. BAJ 2510) could be efficiently extracted from plasma samples with ethyl acetate and analyzed by reverse-phase HPLC with UV detection. Sample acidification improved efficiency, particularly with BAJ 2510. Plasma samples from chronic study rats (taken at week 82) did not yield detectable amounts of spirodiclofen, even at the highest dose level of 2500 ppm. In contrast, dose levels of 50 ppm spirodiclofen in females and 100 ppm in males yielded detectable BAJ 2510. Dose levels of 2500 ppm yielded plasma concentrations of 45 nm/ml (M) and 64 nm/ml (F). The report does not address whether treated rats displayed peaks other than BAJ 2510. Useful supplementary data. No worksheet. Aldous, 1/17/06.

52944-0046 218894 Andersch, I. and J. Köster, "[Dihydrofuranone-3-¹⁴C]BAJ 2740: Investigation of biokinetic behaviour and the metabolism in the rat following subchronic feeding (Supplemental study in support of biokinetics and metabolism in the rat)," Bayer AG, Leverkusen, FRG, Sept. 6, 2000. Bayer Corp. Agric. Div. Report No. 110647. This study evaluated influences of gender and treatment level on metabolism of a single dose of 2 mg/kg

labeled spirodiclofen following 15 weeks of exposure at 50 ppm or 2500 ppm (4 rats/sex/group).

There were no definitive differences between the two treatments. Females excreted substantially more of the enol and much less of the ring hydroxylated products than did males, consistent with the experience of the main metabolism study (Record No. 221358). Useful supplementary data, no DPR worksheet. Aldous, 1/31/06.

52944-0046 221586 Sebesta, C., "An exploratory study to determine the rate and route of elimination of BAJ 2740-dihydrofuranone-3-¹⁴C when administered intravenously or dermally to male Rhesus monkeys." Bayer Corporation, Stilwell, KS, May 3, 2002. Bayer Corp. Agric. Div. Report No. 200110. (See also Record No. 218895, below). This study employed 2 male monkeys, each dosed with about 0.2 mg/kg spirodiclofen in a single treatment. The iv treatment was prepared as a PEG 200 solution in water, and the dermal treatment was a suspension of fine spirodiclofen crystals in water. The patch for the dermal treatment was removed after 8 hr, after which the application area was washed with 1% Ivory detergent solution followed by tape stripping and alcohol swab wiping. Monkeys were maintained in metabolism cages after dosing (except that the first 8 hr after the iv treatment was spent in a primate chair). Following iv dosing, urinary excretion was rapid: 64% of administered dose was obtained in urine within the first 8 hr, with an additional 18% in the next 16 hr. A total of 87% of dose was obtained in urine, and an additional 15% in cage debris/rinse (attributed primarily to urine). About 5% of administered dose was found in feces in the iv test. Measured recovery was slightly more than theoretical. Following dermal treatment, 1.1% of administered dose was found in urine, 0.3% in cage wash, and 0.2% in feces. Most of the dermally administered dose was found in the detergent swab process. An additional 9% was found in the patch or containment dome, and 10% was obtained with the alcohol swab step. Thus the dermal response from this one subject suggested only about 1.6% total absorption. Useful supplementary data, no DPR worksheet. Aldous, 1/31/06.

52944-0046 218895 Wu, Z., "A study to determination the dermal absorption of BAJ 2740-dihydrofuranone-3-¹⁴C in a SC 240 formulation when administered dermally to naïve male Rhesus monkeys." Bayer Corporation, Stilwell, KS, May 3, 2002. Bayer Corp. Agric. Div. Report No. G200109. This study employed 5 male monkeys, each dosed dermally with an average of 0.04 mg/kg spirodiclofen as the SC 240 formulation in a single treatment, considered to represent a plausible field exposure level. Procedures were otherwise much like Record No. 221586, above. About 2.0% of administered dose was recovered in urine plus cage rinse and other collected label attributed to urine. About 0.1% of administered dose was found in feces. Most of the material balance was found in skin wash soap swabs or ethanol extracts of the swabs (>74% of administered dose), plus small additional amounts in the patch, patch securing materials, tape strips, and alcohol swabs. Thus absorption was determined to be about 2.1% of administered dose. Useful supplementary data to estimate plausible worker uptake. No DPR worksheet. Aldous, 1/31/06.

MECHANISTIC STUDIES

NOTE FOR METABOLISM AND MECHANISTIC STUDIES: Spirodiclofen is designated BAJ 2740. The primary initial product of ester cleavage (removing a 2,2-dimethylbutyric acid moiety) is designated BAJ 2510 (or BAJ 2740 enol). Two major products of this enol are the 4-OH and 3-OH addition products to the cyclohexyl ring, i.e. "4-OH BAJ 2510" and "3-OH BAJ 2510." These four entities, for which structures are shown in Document No. 52944-0051, Record No. 218905, page 22; are evaluated in several mechanistic studies. Aldous, 1/4/06.

52944-0048 218899, "Determination of BAJ 2740 and the Enol BAJ 2510 in Plasma and Urine of Dogs in a Chronic Study", (U. Schmidt, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30737, Bayer AG Study No. T 6067346, Bayer Inc., Report No. 110501, 2 February 2001). At week 20, blood samples were taken from 4 dogs per sex at

the high dose (600 ppm) at 0, 2, 4, 7, and 24 hours after feeding. Plasma concentrations of BAJ 2740 and the metabolite BAJ 2510 were evaluated by high performance liquid chromatography (HPLC). BAJ 2740 was below the limit of quantification since it was rapidly cleaved by esterases in plasma and liver to the metabolite BAJ 2510. No other metabolites were identified. Week 20 high dose group mean concentrations of metabolite BAJ 2510 in plasma were 24.8, 17.6, 19.1, 26.7, and 32.4 nmol/ml for males and 26.8, 15.9, 15.8, 25.0, and 28.1 nmol/ml in females at 0, 2, 4, 7, and 24 hours after feeding respectively. BAJ 2510 was also quantified in urine samples taken from 3 female and 1 male high dose (600 ppm) dogs at week 28. One hour after receiving treated diet, dogs were placed in metabolism cages for 5 hours. Urine volumes were 74, 281, and 305 ml in females and 18.6 ml in the male. BAJ 2510 concentrations in urine were 0.46, 0.16, and 0.12 $\mu\text{mol/ml}$ in females and 0.05 $\mu\text{mol/ml}$ in the male respectively. The half life of BAJ 2740 was investigated using spiked rat plasma; it was estimated to be about 15 minutes. Supplemental data. (Green and Leung, 4/3/06).

52944-0051 218905 Freyberger, A., "BAJ 2740 and metabolites: in vitro studies on interactions with microsomal dehydrogenases involved in steroid hormone biosynthesis." Bayer AG, Department of Toxicology, Wuppertal, Dec. 11, 2000. Bayer Corp. Agric. Div. Report No. 110527. This study assessed possible effects of spirodiclofen and three major metabolites (BAJ 2510, 4-OH BAJ 2510, and 3-OH BAJ 2510) on activities of two key enzymes involved in steroid hormone synthesis: 3β -hydroxysteroid dehydrogenase/ $\Delta^{4,5}$ -isomerase (which forms progesterone from pregnenolone) and 17β -hydroxysteroid dehydrogenase (which forms testosterone from androst-4-ene-3,17-dione). Assays were performed in rat testicular microsome preparations. Separations of steroidal compounds were performed by HPLC. Most detections were by spectrophotometry, however androst-4-ene-3,17-dione and testosterone were assayed radiometrically. None of the four test articles inhibited the latter enzyme. Spirodiclofen at 25 and 50 μM were reported to cause 20% and 22% inhibition of 3β -hydroxysteroid dehydrogenase/ $\Delta^{4,5}$ -isomerase. Spirodiclofen at 50 μM caused precipitation. Metabolites did not affect this enzyme activity. Only a single data value per treatment was shown, however variability between values from duplicate assays were noted to be less than 8%. The investigator determined that the observed inhibition might be treatment-related in this *in vitro* environment. It was noted that of the test articles, only BAJ 2510 could be detected in plasma in rat metabolism studies. Thus this observed effect appears not to have relevance in *in vivo* situations. Aldous, no worksheet, 1/6/06.

52944-0051 218906 Schmuck, G., "Effects of BAJ 2740 and its metabolites on the human estrogen and androgen receptor in vitro." Bayer AG, Department of Toxicology, Wuppertal, 10/14/99. Bayer Corp. Agric. Div. Report No. 110535. This study evaluated the effects of spirodiclofen, BAJ 2510, and one of the hydroxy BAJ 2510 metabolites, primarily on hormone response elements in human cell lines (MCF 7 breast cancer cell line or prostate PC-3 cell line for estrogen and androgen receptor, respectively). Hormone response was quantified via luciferase reporter genes using a chemiluminescence reader. In some cases, binding studies for α and β estrogen receptors followed, using a fluorescence polarization technique which could be performed in microtiter wells. At pH's of 6 to 6.5, BAJ 2510 displayed estrogenic and anti-estrogenic activities (Fig. 6). BAJ 2510 bound to α and β estrogen receptors in this pH range (Fig. 7). BAJ 2510 had no effects in the reporter or receptor binding assays at physiological pH (7 or higher). Spirodiclofen and hydroxy BAJ 2510 were uniformly negative in all assays. Thus tested parameters do not reflect spirodiclofen toxicity *in vivo*. Aldous, no worksheet, 1/6/06.

52944-0051 218907 (The first of 2 reports in this record) Freyberger, A., "BAJ 2740 and metabolites: effects on steroidogenesis by rat testicular tissue maintained in dynamic organ culture." Bayer AG, Department of Toxicology, Wuppertal, Dec. 15, 2000. Bayer Corp. Agric. Div. Report No. 110536. Small pieces of rat testes were maintained in a rolling incubator, in Eagle's MEM with supplementary nutrients, and generally in the presence of hCG (to stimulate

steroidogenesis). Testosterone was assayed by RIA after treatments with spirodiclofen (BAJ 2740) or with BAJ 2510, 4-OH BAJ 2510, or 3-OH BAJ 2510. Ketoconazole was used as a reference steroidogenesis inhibitor. BAJ 2510 was the most potent of the spirodiclofen metabolites, sometimes showing inhibition of testosterone secretion with HCG stimulated tissue at 10 μ M. Spirodiclofen was much less potent of an inhibitor, with great variability between trials, but it appeared to provide inhibition at levels of 100 μ M or greater. This variable response was considered by investigators to represent different amounts of hydrolysis of the ester linkage of spirodiclofen, leading to variable amounts of BAJ 2510 as the active inhibitor. The other two metabolites, 4-OH BAJ 2510 and 3-OH BAJ 2510, appeared to be weaker inhibitors, somewhat effective in the range of 100 to 1000 μ M. When this test system was stimulated by dibutyryl cAMP, similar patterns appeared, with BAJ 2510 clearly showing inhibition at 10 μ M, spirodiclofen at 50 μ M, and 4-OH BAJ 2510 and 3-OH BAJ 2510 showing inhibition at 1000 μ M (apparently the only treatment levels tested for the latter two metabolites). Investigators noted that a spirodiclofen disposition study (see Record No. 218908, in the present volume), had found that BAJ 2510 could be quantified in plasma at dietary spirodiclofen dose levels of 100 ppm and above, whereas spirodiclofen itself could not be detected in plasma at any dose levels tested (up to 2500 ppm). Thus it appears that observed inhibition of steroidogenesis should be attributed primarily or entirely to BAJ 2510. Inhibition potency of BAJ 2510 in the range of 10 to 50 μ M approached that of ketoconazole (10 μ M or slightly lower). Useful supplementary data. No worksheet. Aldous, 2/16/06.

52944-0051 218907 (The second of 2 reports in this record) Freyberger, A., "Effects of BAJ 2510 on steroidogenesis: identification of malate dehydrogenase isoenzymes as molecular target." Bayer AG, Department of Toxicology, Wuppertal, Jan. 25, 2001. Bayer Corp. Agric. Div. Report No. 110537. Testicular mitochondrial preparations were evaluated for side chain cleavage of 25-OH cholesterol to pregnenolone (by assaying for progesterone after a subsequent oxidation step). In a mitochondrial preparation supplemented with NADP and in an environment of low malate levels (0.5 mM); 100 μ M and 300 μ M BAJ 2510 reduced progesterone synthesis to 68% and 24% of control groups, respectively. In contrast, spirodiclofen, 4-OH BAJ 2510, and 3-OH BAJ 2510 at concentrations up to 100 μ M or (in the case of spirodiclofen, at the limits of solubility) had little or no effect on progesterone synthesis. When 0.5 mM citrate (and no malate) was present as a substrate (citrate also being capable of reducing NAD), even 300 μ M BAJ 2510 had no remarkable effect on progesterone synthesis. This suggested an interference of BAJ 2510 with the Krebs cycle related to malate dehydrogenase activity. This was confirmed when investigators evaluated the oxidation of NADH due to malate dehydrogenase activity (assessing activity from both mitochondrial and cytoplasmic fractions): there was a clear dose-responsive inhibition of such activity due to BAJ 2510 concentrations in the 1 to 100 μ M range (mitochondrial) or the 10 to 300 μ M range (cytoplasmic). In contrast, BAJ 2510 had no effect on malic enzyme activity (assessed by NADP reduction with malate as substrate). In a dynamic organ culture of testicular tissue (6 hr incubation with steroidogenesis stimulated by 1 IU/ml hCG: for methods, see the other report in this DPR Record), BAJ 2510 concentrations of 10 to 300 μ M caused marked, dose-related decrements in testosterone in both the tissue pieces and in the medium. As noted above in this paragraph, an early step in progesterone synthesis from 25-OH cholesterol was markedly inhibited by BAJ 2510 in mitochondrial preparations. In contrast, progesterone levels were not statistically significantly reduced at any level with BAJ 2510 in the dynamic organ culture system with testicular tissue. As a positive control, ketoconazole profoundly reduced testosterone in tissues and medium, also without significantly reducing the quantity of progesterone in the tissue pieces. Thus it appears that BAJ 2510 toxicity is related to interference with cellular energy metabolism. Useful supplementary data. No worksheet. Aldous, 1/17/06.

52944-0051 218909 Schmidt, U., "BAJ 2740: Determination of BAJ 2740 and the enol BAJ 2510 in plasma: influence on the concentration of cholesterol and triglyceride in adrenals and

liver of rats in a mechanistic subacute study" (Revised Final Report). Bayer AG, Department of Toxicology, Wuppertal, 2/23/2001, Bayer Corp. Agric. Div. Report No. 110539. Ten male Wistar rats/group were dosed in diet for 4 weeks with 0, 1000, or 5000 ppm spirodiclofen. Blood samples were taken after 1 day and after 4 weeks of treatment for analysis of spirodiclofen and of BAJ 2510. Liver and adrenal gland samples were processed for concentrations of cholesterol and triglycerides at termination. No spirodiclofen was detected in plasma at either time. BAJ 2510 concentrations were comparable after 1 day or 4 weeks of exposure for 1000 and 5000 ppm: 56 and 119 nmol/ml after 1 day, and 35 and 97 nmol/ml after 4 weeks, respectively. Adrenal cholesterol at 4 weeks for respective 0, 1000, and 5000 ppm groups was 18, 37, and 78 μ mol/g (strong dose-response). Triglycerides showed a modest but statistically significant increase at 5000 ppm (respective levels of 42, 42, and 56 μ mol/g). There were no significant changes in cholesterol nor in triglycerides in liver. The investigator justifiably asserted that the great increase in cholesterol in the adrenal glands (presumed by this reviewer but not specified by investigators to reflect the cortex) could reflect an "excess storage of unmetabolized steroid precursors." Useful supplementary data. No worksheet. Aldous, 1/18/06.

52944-0051 218910 Schmidt, U., "BAJ 2740: influence on the concentration of α -tocopherol, ubiquinone and dolichols in a special subacute dog study." Bayer AG, Department of Toxicology, Wuppertal, 2/20/01, Bayer Corp. Agric. Div. Report No. 110540. Five male beagles per group were dosed in diet with 0, 100, or 2000 ppm spirodiclofen for 8 weeks. This study evaluated the effects of spirodiclofen on several key products based on isoprene units. Investigators measured plasma ubiquinone and α -tocopherol, liver ubiquinone and α -tocopherol, dolichols and dolichyl phosphates in testes, and GSH in liver. Plasma ubiquinone and α -tocopherol were reduced to 64% and to 42% of controls, respectively, after 7 weeks at 2000 ppm, with no changes at 100 ppm. At week 8 termination, 2000 ppm group liver ubiquinone was non-significantly reduced to 86% of control concentration, however α -tocopherol concentration was reduced to 46% of control. There were small (non-significant) reductions in concentrations of dolichols and dolichyl phosphates in testes, without systematic changes in the distribution of isoprene number ranges of either product. GSH was uniformly elevated in liver tissues of both treatment groups, likely an incidental finding. The investigator considered the responses consistent with a reduction of activity of 3-hydroxy-3-methylglutaryl CoA reductase, which synthesizes a common precursor to several key products, including cholesterol, ubiquinones, and dolichols. This study did not investigate effects on this enzyme itself. Since α -tocopherol is not synthesized by dogs (or humans), the marked plasma reductions in α -tocopherol were attributed to hampered lipid transport capacity. Useful supplementary data. No worksheet. Aldous, 1/20/06.

52944-0051 218911 Freyberger, A., "2,4-Dichloromandelic acid (BAJ 2740 metabolite): in vitro studies on interactions with steroidogenesis using rat testicular tissue." Bayer AG, Department of Toxicology, Wuppertal, 3/23/01. Bayer Corp. Agric. Div. Report No. 110541. [NOTE: test article is α -hydroxy-2,4-dichlorophenyl acetic acid, stated to be a main metabolite in plants]. The investigator evaluated the test article for testosterone release into the medium in two trials, using the dynamic organ culture for testicular tissue described in Record No. 218907 (Bayer Report No. 110536). There was no dose-response, nor any reproducible change at any exposure level. A third and final trial included assays for testosterone in medium and in the tissue. This trial was also negative. Ketoconazole was a reliable positive control in all trials. Useful supplementary data. No worksheet. Aldous, 1/20/06.

52944-0052 218912, "Inhibition of Cholesterol Esterase by BAJ 2740 *In Vitro*", (A. Freyberger, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30529, Bayer Inc., Report No. 110542, 6 November 2000). A radiometric assay was performed to determine the effect of BAJ 2740 and its main plasma metabolite BAJ 2510 (BAJ 2740 enol) on

cholesterol esterase *in vitro*. Commercially available pancreatic cholesterol esterase was used. Inhibition of cholesterol esterase was determined by incubating BAJ 2740 (98.6%) at 0 (ethanol), 0.1, 1, 10, 30, and 100 μ M, cholesterol esterase (5 μ g/ml), sodium taurocholate (3 mg/ml), and cholesterol [$1\text{-}^{14}\text{C}$] oleate (3.3 to 7.5 μ M) together in 0.2 M phosphate buffer (pH 6.6) for 10 minutes at 37°C in a total volume of 0.5 ml. Incubations (performed in triplicate) were initiated by addition of cholesterol [$1\text{-}^{14}\text{C}$] oleate. Incubations were stopped by addition of 50 μ l of 1 M HCl and extracted with 1 ml heptane/isopropanol (2:1 v/v) and with 1 ml heptane (twice). Extracts were dried (stream of nitrogen) and residue was dissolved in 400 μ l heptane/isopropanol. Radiolabel contained in the extracted aqueous phase and in aliquots from the organic extract were measured by liquid scintillation counting (LSC). Cholesterol [$1\text{-}^{14}\text{C}$] oleate was separated from enzymatically released [$1\text{-}^{14}\text{C}$] oleic acid by thin layer chromatography (TLC). BAJ 2740 dose-dependently inhibited cholesterol esterase. The assay was repeated using BAJ 2510 at 0 (ethanol), 0.1, 1, 10, 30, 100, 300, and 1000 μ M. BAJ 2510 did not inhibit cholesterol esterase at 100 μ M and showed weak inhibition at 1000 μ M. An additional assay was performed to determine whether BAJ 2740 was an alternate/competing substrate for cholesterol esterase. BAJ 2740 in ethanol at 5 and 50 μ M was incubated with cholesterol esterase in duplicate for 10 minutes at 37°C. Acidification with 50 μ l 1 M HCl and addition of 0.5 ml acetonitrile stopped incubation. Samples were centrifuged and the supernatant was analyzed for BAJ 2740 by High Performance Liquid Chromatography (HPLC). Recovery of BAJ 2740 from the enzymatic incubation mixtures was similar or comparable to recovery from controls lacking enzyme or containing heat-inactivated enzyme at the end or the incubation period. Also, no peak with retention time corresponding to that of BAJ 2740, the product of ester cleavage, was observed. BAJ 2740 was not considered an alternate/competing substrate for the enzyme. Supplemental. (Green and Leung, 4/4/06).

52944-0052 218913, "BAJ 2740, Special Study for Subchronic Oral Toxicity in Rats (Hormone Determinations in Female Rats, Feeding Study for 19 Weeks and 11 Weeks Recovery)", (P. Andrews, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30872, Bayer AG Study No. T 9069383, Bayer Inc., Report No. 110544, 23 March 2001). 15 female Wistar Hsd Cpb: WU rats per group received BAJ 2740 (97.5% spirodiclofen) in the diet (containing 1% peanut oil) at 0, 2500, and 10000 ppm for 19 weeks followed by an 11-week recovery period receiving untreated standard diet. BAJ 2740 intakes were 242.4 and 1209.6 mg/kg/day at 2500 and 10000 ppm respectively during the treatment period. There were no treatment-related deaths (one high-dose animal was humanely sacrificed in recovery week 4 due to eye damage from a blood sampling error). 7/15 and 15/15 females had light-colored feces during the treatment period at 2500 and 10000 ppm respectively. Group mean bodyweights were reduced 2% to 3.7% at 2500 ppm and 6.6% to 12.7% at 10000 ppm during the treatment period relative to controls. Group mean food consumption (g/kg/day) was increased 9.7% and 36.8% at 2500 and 10000 ppm respectively compared to controls. Both parameters reversed during the recovery phase. Testosterone, luteinizing hormone, estradiol, and progesterone were determined at 7, 9, 11, 13, 17, and 19 weeks of treatment and during recovery weeks 2 and 6. Dose-dependent decreases in testosterone, estradiol and progesterone levels were noted at both treatment levels with the greater effect on progesterone. Subsequently, a significant difference in the estradiol:progesterone ratio was recorded in weeks 13 and 17 of treatment. Hormone levels were normal after 2 and 6 weeks of recovery. At necropsy (performed after the recovery period), increased uterus size was noted in 4/14 high dose animals and group mean relative adrenal weights (mg/100g) were significantly increased at the high dose level. Supplemental. (Green and Leung, 4/5/06).

52944-0052 218914, "BAJ 2740, General Pharmacological Study", (H. Takahashi, The Institute of Environmental Toxicology, Japan, Bayer Inc., Report No. 110830, 13 March 2000). The study was performed to evaluate and characterize acute poisoning with BAJ 2740 in order to provide information for treatment. 3 ICR mice per sex per group received a single

intraperitoneal injection (i.p.) of BAJ 2740 (97.8% spirodiclofen) at 0 (1% Tween 80 aqueous solution), 128, 320, 800, 2000, and 5000 mg/kg. Bodyweights were measured and clinical signs were observed 0.5, 3, and 6 hours, and 1, 2, 3, and 7 days after treatment. Two females at 800 mg/kg, all females at 2000 mg/kg, and all males and females at 5000 mg/kg died within 2 days. At 800 mg/kg and above in females, and, at 2000 mg/kg and higher in males; awareness, motor activity, motor coordination, muscle tone, reflexes, grip strength, and autonomic signs were decreased at 30 minutes post-dosing. Animals appeared normal at 3 days post-dosing. There was no effect on bodyweight. Separately, 5 male Sprague-Dawley rats per group received a single oral gavage dose of BAJ 2740 at 0, 2000, and 5000 mg/kg. Cageside observations were performed 1 and 6 hours, and 1, 2, 3, and 7 days post-dosing (bodyweights were also measured on days 1, 2, 3, and 7). Body temperature, pupil size, and grip strength were also evaluated at each time point. No treatment-related effects were reported. In another trial, 8 male ICR mice per group received a single i.p. dose of BAJ 2740 at 0, 51.2, 128, 320, 800, 2000, and 5000 mg/kg, subsequently, hexobarbital sleeping time was measured. One day after dosing, each animal received a single subcutaneous injection of hexobarbital (100 mg/kg) and the time to regain the righting reflex was measured. 3 animals each at 2000 and at 5000 mg/kg died prior to the hexobarbital injection, and, within 3 hours after hexobarbital injection, one animal at 2000 mg/kg and 4 at 5000 mg/kg died. No effects were observed at 800 mg/kg and below. In a subsequent trial, 5 male Sprague-Dawley rats per group received an oral gavage dose at 0, 2000, and 5000 mg/kg. Systolic blood pressure and heart rates were measured pre-treatment, and 1 and 6 hours, and 1, 2, 3, and 7 days after dosing. No treatment-related effects were reported. Additionally, 5 male Sprague-Dawley rats per group received a single oral dose at 0, 2000, and 5000 mg/kg followed by urinalysis. Physiological saline (25 ml/kg) was given orally one day post-dosing followed by urine collection (in metabolism cages) for 3 hours. No treatment-related urine effects were recorded. In another trial, clinical chemistry parameters were determined for 5 male Sprague-Dawley rats 24 hours after a single oral dose at 0, 2000, and 5000 mg/kg. No treatment-related blood effects were recorded. Also, effects of BAJ 2740 on transport activity in the small intestine were measured in 8 male ICR mice per group that received a single i.p. dose at 0, 51.2, 128, 320, 800, 2000, and 5000 mg/kg. Mice were fasted for 16 hours pre-treatment (with free-access to water). Charcoal suspension was administered orally (10 ml/kg) 24 hours post-dosing. Mice were euthanized 30 minutes later and the mobility of the charcoal was calculated by comparing the distance the charcoal was transported in the intestine relative to the total length of the intestine. 3 animals died at 5000 mg/kg prior to charcoal administration. At 320 and 800 mg/kg, intestinal transport increased, at 2000 mg/kg it was comparable to controls, and at 5000 mg/kg it decreased. At 128 mg/kg and below, intestinal transport was comparable to controls. Supplemental. (Green and Leung, 4/4/06).

52944-0052 221261, "BAJ 2740 and Metabolites, *In Vitro* Studies on Interactions with Microsomal Monooxygenases Involved in Steroid Hormone Biosynthesis", (A. Freyberger, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30594, Bayer Inc., Report No. 110543, 11 December 2000). This study was performed to evaluate potential inhibitory effects of BAJ 2740 and its metabolite BAJ 2510 on microsomal cytochrome P-450-dependent monooxygenases (17 α -monooxygenase and C-17, 20-lyase) involved in steroid hormone synthesis. In one assay, progesterone (5 μ M), rat testicular microsomal preparation (1 mg/ml), BAJ 2740 (98.6%) at 0 (DMSO) and 50 μ M (precipitate was reported) and BAJ 2510 (BAJ 2740-Enol) (98%) at 0 and 300 μ M were preincubated in duplicate for 2 minutes in potassium phosphate buffer (0.1 M; pH 7.4). 500 μ M NADPH were added and the mixtures incubated at 37°C for 20 minutes. At 20 minutes, acetonitrile (ice-cold) was added (to stop incubation) and samples were centrifuged and the resulting supernatants analyzed by high performance liquid chromatography for 17 α -monooxygenase, 17 α -hydroxyprogesterone,

androstenedione, and testosterone. 17 α -monooxygenase activity was not inhibited by BAJ 2740 and BAJ 2510 *in vitro*. Formation of 17 α -hydroxyprogesterone from progesterone was observed. In another assay to determine effects on C-17 and 20-lyase, 17 α -hydroxyprogesterone (5 μ M), coumestrol (50 μ M) (to inhibit 17 β -hydroxysteroid dehydrogenase), rat testicular microsome preparation (1 mg/ml), BAJ 2740 (98.6%) at 0 (DMSO), and 50 μ M (precipitate was reported) and BAJ 2510 (BAJ 2740-Enol) (98%) at 0 and 300 μ M were preincubated in duplicate for 2 minutes in potassium phosphate buffer (0.1 M; pH 7.4). Following addition of NADPH (500 μ M) and incubation at 37°C for 40 minutes, samples were centrifuged and supernatants analyzed (HPLC) for C-17, 20 lyase, and androstenedione. C-17 and 20-lyase were slightly inhibited by BAJ 2740 and BAJ 2510 and formation of androstenedione from 17 α -hydroxyprogesterone was observed. Ketoconazole was used as positive control in both assays. Supplemental. (Green and Leung, 4/4/06).

52944-0053 218919 Wahle, B. S., "Technical grade BAJ 2740: A special toxicity testing study to determination the liver enzyme activity profile in the mouse," Bayer Corporation, Stilwell, KS, and Bayer AG, Wuppertal, Germany, 9/30/02. Bayer Corp. Agric. Div. Report No. 200291. There were 10 mice/sex/group at 0, 500, 3500, and 7000 ppm spirodiclofen (97.1% purity) in diet (equivalent to 87, 640, and 1199 mg/kg/day in M and 107, 774, and 1665 mg/kg/day in F). In-life measures included body weights, food consumption, and clinical signs. At termination, investigators assessed liver weights and liver activities of 7-ethoxycoumarin deethylation (ECOD), 7-ethoxyresorufin deethylation (EROD), aldrin epoxidase (ALD), epoxide hydrolase (EH), glutathione-S-transferase (GS-T), and UDP-glucuronyltransferase (GLU-T). Testes and adrenals of control and 7000 ppm rats were assayed for mRNA contents corresponding to nine enzymes associated with steroid hormone production. These mRNA studies used tissues from the same rats which were addressed above, but were performed in Wuppertal. The mRNA segment is part of the same Bayer report and the same DPR record described above, although such testing was not specified in the report title. Body weights were not affected by treatment. Food consumption was statistically reduced in all treated male groups during week 1, and in 500 ppm and 3500 ppm females during week 1. In all cases food consumption was unaffected at week 2. There were substantial increases in liver activities of ECOD, EROD, and ALD activities in 3500 ppm and 7000 ppm males. Females at these dose levels had dose-related and significant increases in ECOD and GS-T. In addition, 7000 ppm females had substantial increases in EROD, ALD, and EH. Levels of P450 side chain cleavage enzyme 11a1 mRNA (Cyp11a) were significantly increased in testes and adrenals of M and F, consistent with induction of this early step in the common pathway for steroid hormone products of both organs. Cytochrome P450 17-alpha hydroxylase/C17-20 lyase mRNA was increased significantly in testes and in adrenals of females only. The associated enzyme plays a role in adrenal and gonadal steroid hormone production. Steroid-11-beta-hydroxylase (Cyp11b2) was appreciably increased in adrenals of both sexes, but was not detectable in testes. This is consistent with the presence of 11-hydroxylation in adrenal steroids like aldosterone, but not in most gonadal hormones like testosterone. Cytochrome P450 XXI (steroid 21 hydroxylase) (Cyp21a1) was significantly elevated in adrenals of males, and non-significantly elevated in testes: this enzyme appears to be most relevant to production of adrenal corticoids (which typically have hydroxyls on C-21), although there was much more of this mRNA in testes than in adrenals. These studies indicate that a mode of action of spirodiclofen involves changes in activities of key enzymes in liver, adrenals, and testes, although the pivotal events governing the observed changes are not specified. Useful supplementary data. Aldous, Feb. 6, 2006.

52944-0053 218918 Freyberger, A., "BAJ 2510 (BAJ 2740 enol): Effects on rat testicular mitochondrial NADH (reduced nicotinamide adenine dinucleotide) and NADPH (reduced nicotinamide adenine dinucleotide phosphate) levels," Bayer CropScience AG, Wuppertal, Germany, 9/12/02. Bayer Corp. Agric. Div. Report No. AT00015. Testes from 10 to 13-week old Wistar rats were homogenized in chilled buffer, then the mitochondrial fraction was isolated

by centrifugation, and re-suspended for immediate use. To determine the influence of supplementary malate and NADP on cholesterol side-chain cleavage, mitochondrial preparations were incubated in a medium containing NADH, malate (usually 5 mM), 25-hydroxycholesterol, and NADP. This reaction was stopped by addition of cholesterol oxidase, followed by HPLC separation and quantification of pregnenolone (indicative of the extent of side chain cleavage). Cholesterol side chain cleavage rate was rapid for up to about 3 minutes, then slowed down considerably. For this reason, incubations for the main study tests were of short duration. Under the above conditions, the reaction was very dependent on malate (only 8-10% of control cleavage occurred without malate). Presence or absence of supplementary NADP was of minimal or no importance in side chain cleavage. The majority of tests assessed the effect of BAJ 2510 on NADH and NADPH concentrations in mitochondrial preparations. Mitochondria were incubated in media containing 25-hydroxycholesterol and malate (0.05 mM, 0.5 mM, or 5 mM). The reaction was stopped by adding respiratory inhibitors, disrupting mitochondrial membranes with desoxycholic acid, and rapidly heating the mixtures to destroy enzymes which might affect levels of NADH and NADPH (these reduced nucleotides being thermally stable under these conditions). NADH and NADPH were then quantified by HPLC. A known inhibitor of malic enzyme (tartronate) served as an effective positive control. Investigators determined that BAJ 2510 was a competitive inhibitor of malate dehydrogenase, which is somewhat consistent with the data: in the presence of 5 mM malate, there was no measurable inhibition by BAJ 2510, whereas at 0.05 or 0.5 mM BAJ 2510, there was a highly significant reduction of NADPH (to about 70% of control) and of NADH (to about 60% of control) (equivalent responses for 0.05 and 0.5 mM BAJ 2510 in this range). In one test, malate was inadvertently not added to the preparation, in which case NADH and NADPH concentrations were 53% and 59% of controls, respectively. This suggests that exogenous malate is not critical to maintenance of nicotinamide-reducing equivalents. This study supports but does not prove the postulate that spirodiclofen administration leads to testicular (and by inference adrenal) toxicity by metabolism to BAJ 2510, which competes with malate dehydrogenase, leading to a critical reduction of NADH and NADPH concentrations in target cells: the lack of adequate NADPH in turn is proposed to be sufficient to impede side-chain cleavage of cholesterol so that cells cannot produce sufficient steroid hormones to function properly, and are over-stimulated by feedback loops, leading to vacuolation, hypertrophy, and in the case of rats, to gonadal tumors. It is out of the scope of the present study to demonstrate that BAJ 2510 levels obtained *in vivo* are sufficient to cause these perturbations by this mechanism. Useful supplementary data. Aldous, Feb. 9, 2006.

52944-0053 218918 (continued with record number): Bayer Corp. Agric. Div. Report No. AT00015-1. This is an amendment to the cited record, with 2 pages showing corrections.

52944-0053 218917 Christenson, W. R. *et al.*, "The development and integration of a toxicological mode of action (MOA) into the hazard (and risk) assessment for a proposed agrochemical (BAJ 2740) with endocrine disrupting properties including evidence of a carcinogenic potential," 4/22/02, Bayer Corp. Agric. Div. Report No. 111009. This report applies to risk assessment for spirodiclofen, citing mechanistic studies and FIFRA studies which the authors conclude support a threshold-based endpoint for oncogenicity assessment for spirodiclofen. Aldous, no worksheet (no new data in this report), 2/16/06.

STUDIES ON METABOLITES

Mutagenicity Studies

**52944-0042 218889, "BAJ 2740-KETOHYDROXY, *Salmonella*/Microsome Test Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30671, Bayer AG Study No. T 8069887, Bayer Inc., No. 110532, 29 January 2001). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to BAJ 2740-ketohydroxy (98.5% purity), in

the presence and absence of S9, at 0 (ethanol), 16, 50, 158, 500, 1581, and 5000 µg/plate in the first trial and at 0, 5, 10, 20, 40, 80, 160, and 320 µg/plate in a repeat trial. Treatment was for 48 hours at 37°C after plate incorporation. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated for 20 minutes (37°C) in test tubes prior to plate incorporation. No increases in the number of revertants per plate at any level were indicated. Strain specific bacterial toxicity (reduced titer) was recorded at 158 µg/plate and higher in Trial One and at 80 µg/plate and above in Trial Two. Supplemental to data for BAJ 2740. (Green and Leung, 4/12/06).

52944-0042 220241, "C6-Hydroxyester, *Salmonella*/Microsome Test, Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. 31003 and 31003A, Bayer AG Study No. T0070102, Bayer Inc., Report No. 110824 and 110824-1, 8 May 2001). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to C6-hydroxyester (99.7% purity), in the presence and absence of S9, at 0 (DMSO), 50, 158, 500, 1581, and 5000 µg/plate for 48 hours at 37°C. Two trials were performed. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated in test tubes for 20 minutes (37°C) prior to plating. No increases in the number of revertants per plate at any level were indicated. In Trial 2 (preincubation), the bacterial lawn was reduced at 5000 µg/tube in the absence of S9. Supplemental to BAJ 2740. (Green and Leung, 4/12/06).

52944-0042 220242, "BAJ 2740-Hexylester, *Salmonella*/Microsome Test, Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 31564, Bayer AG Study No. T 3070501, Bayer Inc., Report No. 110946, 30 November 2001). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to BAJ 2740-Hexylester (95.5% purity), in the presence and absence of S9, at 0 (DMSO), 16, 50, 158, 500, 1581, and 5000 µg/plate for 48 hours at 37°C. Two trials were performed. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated in test tubes for 20 minutes (37°C) prior to plating. No increases in the number of revertants per plate at any level were indicated. Strain specific bacterial toxicity (reduced titer) was recorded at 158 µg/plate and higher in Trial One. Precipitate was recorded at 5000 µg/plate (tube) in both trials. Supplemental to BAJ 2740. (Green and Leung, 4/12/06).

52944-0041 220246, "BAJ 2740-Enol (Metabolite of BAJ 2740), *Salmonella*/Microsome Test, Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH-28631, Bayer AG Study No. T 9059933, Bayer Inc., Report No. 110505, 7 April 1999). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to BAJ 2740-Enol (98% purity), in the presence and absence of S9, at 0 (DMSO), 16, 50, 158, 500, 1581, and 5000 µg/plate for 48 hours at 37°C. BAJ 2740-Enol is a metabolite of BAJ 2740. Two trials were performed. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated for 20 minutes (37°C) prior to plating. There was no increase in the number of revertants per plate at any level. Positive controls were functional. Strain specific bacterial toxicity (reduced titer) was recorded at 500 µg/plate and higher in Trial One and at 5000 µg/plate in Trial Two. Supplemental to BAJ 2740 data. (Green and Leung, 4/12/06).

52944-0041 220248, "BAJ 2740-MA-3OH-Cyclohexylester, *Salmonella*/Microsome Test Plate Incorporation and Preincubation Method", (B. Herbold, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30683, Bayer AG Study No. T 2069926, Bayer, Inc. Report No. 110530, 30 January 2001). *Salmonella typhimurium* LT2 strains TA98, TA100, TA102, TA1535, and TA1537 were exposed (in triplicate) to BAJ 2740-MA-3OH-cyclohexylester

(98.6% purity), in the presence and absence of S9, at 0 (DMSO), 16, 50, 158, 500, 1581, and 5000 µg/plate for 48 hours at 37°C. Two trials were performed. The direct plate incorporation method was used in the first trial. In the second trial, cultures were preincubated for 20 minutes (37°C) prior to plating. No increases in the number of revertants per plate at any level were indicated. Positive controls were functional. Strain specific bacterial toxicity (reduced titer) was recorded at 50 µg/plate and higher in Trial One. Supplemental to BAJ 2740 data. (Green and Leung, 4/12/06).

SUBCHRONIC STUDIES

Rat 4-Week Dietary Toxicity Study

0050, 218903; "BAJ 2740 Study for Subacute Oral Toxicity in Rats (Feeding Study for 4 Weeks) (Revised Report to Bayer AG Report No. 26371)" (Krötlinger, F. and Geiss, V., Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30460, Bayer AG Study No. T 4058371, 11/17/00). BAJ 2740 (Batch no. NNL 5605-02, purity = 98.2%) was admixed to the diet (containing 1% peanut oil) and fed to 5 Wistar (Hsd Cpb:WU) female rats per dose at dose levels of 0, 100, 500, or 5000 ppm (0.0, 10.0, 49.6, and 569.3 mg/kg/day, respectively) for 4 weeks. No mortalities occurred. No clinical signs were observed. No effect on body weight was observed. No effect on hematological parameters was observed. Treatment-related increases in aspartate aminotransferase and alanine aminotransferase levels were observed at 5000 ppm and treatment-related decreases in cholesterol, triglycerides, and protein levels were observed at 5000 ppm. A treatment-related increase in activity of the liver enzyme 7-ethoxycoumarin deethylase at 500 and 5000 ppm was observed. No treatment-related effect on organ weights was observed. Microscopic examination of liver tissue revealed no treatment-related effects. **No adverse effects.** NOEL (F) = 49.6 mg/kg/day (500 ppm) based on elevated liver enzymes levels. **Supplemental study** (only female animals were used, only 5 animals per dose level were used, and the test animals were treated for only 4 weeks). (Corlett, 01/04/06)

Rat Subchronic Dietary Toxicity Study

0029, 218871; "BAJ 2740 Study on Subchronic Toxicity in Wistar Rats (Administration in Food Over 14 Weeks with a 4 Week Recovery Period)" (Wirnitzer, U. and Romeike, A., Bayer AG, Wuppertal, Germany, Bayer AG Report No. PH-27186, Bayer AG Study No. T2060691, 01/28/98). 821. BAJ 2740 (Batch no. NNL 5605-7-8, purity = 99.1%) was admixed to the diet (containing 1% peanut oil) and fed to 10 Wistar rats per sex per dose at dose levels of 0, 100, 500, 2500, or 12500 ppm (0, 2.1, 10.3, 51.0, and 239.3 mg/kg/day, respectively for males and 0, 1.6, 9.0, 40.7, and 174.0 mg/kg/day, respectively for females) for up to 14 weeks [with 10 additional rats per sex per dose level at 0 and 12500 ppm dose levels to test recovery (4-week recovery period used)]. Also, for immunotoxicological investigations, satellite groups of 5 rats per sex per dose were likewise administered dose levels of 0, 100, 500, 2500, and 12500 ppm for 4 weeks at which time the investigations were conducted. No mortalities occurred. No dose-related clinical signs were observed. A treatment-related decrease in mean body weight was observed in both sexes at 12500 ppm with no recovery observed in both sexes. A treatment-related increase the mean thromboplastin time and a treatment-related decrease in mean leukocyte count in both sexes was observed in both sexes at 12500 ppm with recovery observed in both sexes. A treatment-related decrease in mean platelet level was observed in males at 12500 ppm with recovery observed. Treatment-related decreases in mean cell volume and mean corpuscular hemoglobin and a treatment-related increase in mean erythrocyte level were observed in females at 12500 ppm with no recovery observed in any of these parameters. Treatment-related increases in aspartate aminotransferase (both sexes), alanine aminotransferase (females only), and alkaline phosphatase (both sexes) were observed at 12500 with recovery observed in females but not males. Treatment-related decreases in cholesterol (both sexes), triglycerides (both sexes), bilirubin (both sexes), and protein (males only) levels were observed at 12500 ppm with recovery observed in females but not males (except for the

bilirubin level where recovery was observed). A treatment-related increase in mean relative adrenal gland weights in both sexes at 12500 ppm was observed with recovery observed in both sexes. Microscopic examination revealed a treatment-related increase in severity of adrenal glands with cortical vacuolation (uniformly small and densely packed) in both sexes at 2500 and 12500 ppm (recovery observed at 12500 ppm in both sexes) and in females at 500 ppm and treatment-related vacuolation of the mucosal epithelium of the small intestine (primarily the jejunum) in both sexes at 2500 and 12500 ppm (recovery was observed in both sexes at 12500 ppm). **No adverse effects.** NOEL (M) = 10.3 mg/kg/day (500 ppm) and NOEL (F) = 1.6 mg/kg/day (100 ppm) based on microscopic findings discussed above. **Acceptable.** (Corlett, 12/16/05)

Mouse Subchronic Dietary Toxicity Study

0028, 218870; "BAJ 2740 Study on Subchronic Toxicity in CD-1 Mice (Administration in the Feed Over 13 Weeks)" (Leser, K.H. and Romeike, A., Bayer AG, Wuppertal, Germany, Bayer AG Report No. 26536, 07/01/97 and Bayer AG Report No. 26536 A, 06/09/98, both part of Bayer AG Study No. T7060885). 821. BAJ 2740 (Batch no. NNL 5605-7-8, purity = 99.1%) was admixed to the diet (containing 1% peanut oil) and fed to 10 Crl:CD-1(ICR)BR mice per sex per dose at dose levels of 0, 100, 1000, or 10000 ppm (0, 15.3, 163.8, and 1640.1 mg/kg/day, respectively for males and 0, 30.1, 233.6, and 2685.2 mg/kg/day, respectively for females) for approximately 13 weeks. No mortalities occurred. No treatment-related clinical signs were observed. No effect on body weight was observed. Statistically significant decreases in mean hemoglobin and mean cholesterol levels were observed in females at 10000 ppm. No toxicologically significant changes in organ weights were observed. Necropsy revealed no treatment-related abnormalities. Microscopic examination revealed treatment-related hepatocyte hypertrophy in males at 100, 1000, and 10000 ppm and in females at 10000 ppm, periportal cytoplasmic vacuolation in both sexes at 10000 ppm, zona fasciculata vacuolation in the adrenal glands in males at 10000 ppm and in females at 1000 and 10000 ppm, and minimal to moderate hypertrophy of the Leydig cells at 1000 and 10000 ppm and minimal to slight vacuolation of the Leydig cells in the testes at 10000 ppm. **Possible adverse effect indicated:** Leydig cell hypertrophy and vacuolation in the testes. NOEL (M) not determined (hepatocyte hypertrophy observed at all dose levels) and NOEL (F) = 30.1 mg/kg/day (100 ppm) based on zona fasciculata vacuolation in the adrenal glands. **Supplemental study** (no ophthalmological examinations conducted on the eyes of the test animals). (Corlett, 01/20/06)

Dog 4-Week Dietary Toxicity Study

52944-0048, 0049 218900 and 218901, "BAJ 2740, Subacute Toxicity Study in Beagle Dogs (Dose Range-Finding Study by Feed Admixture Over 4 Weeks) (Revised Report to Bayer AG Report No. PH 29421)", (H. Wetzig, A. Romeike (pathologist original study), and E. Sander (consulting pathologist revised study), Bayer AG, Department of Toxicology, Wuppertal, Germany, Original Bayer AG Report No. PH 29421, Revised Bayer AG Report No. PH 31338, Original Bayer Inc., Report No. 110502, Revised Bayer Inc., Report No. 110502-1, original report date: 9 December 1999; revised report date: 24 August 2001). 2 Beagle dogs per sex per group received BAJ 2740 (99.1% spirodiclofen) in the diet (moistened 1:1 with water) at 0 (standard diet), 400, 2000, and 10000 ppm for 28 days. Each animal received 350 g of food per day. Group mean BAJ 2740 intake was 10.4, 60.1, and 312.5 mg/kg/day for males and 12.1, 68.5, and 284.9 mg/kg/day for females at 400, 2000, and 10000 ppm respectively during the 28-day treatment period. All animals survived to termination. Clinical signs, pulse rates, reflexes, behavior, food and water intake, bodyweight, hematology, and urinalyses were not affected by treatment. At 2000 ppm, alanine aminotransferase activity was slightly increased in one male and one female at weeks 2 and 4. At 10000 ppm, alanine aminotransferase (ALAT) activity was strongly increased, especially in males, followed by time-dependent increases in aspartate aminotransferase (ASAT), alkaline phosphatase (APh), and glutamate dehydrogenase (GLDH). At week 4, dose-dependent decreases in triglycerides (TRIGL) and thyroxine (T4) were recorded at 2000 ppm and higher. Cholesterol, protein, albumin, and iron values were reduced at 10000 ppm. Activities of phase I liver enzymes N- and O-demethylase and P-450 were

increased at 2000 ppm and higher. 7-ethoxycoumarin deethylase (ECOD) was dose-dependently induced at 2000 ppm and above in both sexes. The increase was more pronounced in males. Aldrin epoxidase (ALD) was slightly induced at the high dose in both sexes. The phase II enzyme, UDP-glucuronyltransferase (GLU-T), was slightly induced in mid and high dose males. Dose-dependent increases were noted for group mean relative liver, kidney, and adrenal weights in both sexes at the mid and high dose levels. Adrenal weights were also increased for females at 400 ppm. Minimal to slight periportal single cell necroses in livers of all high dose animals was recorded at microscopy. Vacuolation of Leydig cells was found in the testes of males at 2000 and 10000 ppm. Additionally, one high dose male had hypertrophy/activation of the Leydig cells. Testes and prostate of the animal were slightly immature and the epididymides showed a massive oligo/aspermia and slight spermatic debris. In adrenal glands, increased cytoplasmic vacuolation in the cortex was observed at the mid and high dose levels in both sexes. In the jejunum of mid and high dose animals, moderate to marked vacuolation of superficial mucosal epithelial cells was noted. NOEL = 400 ppm (Based on liver enzyme activity and testes and adrenal histopathology). This was not a FIFRA guideline study. Data are supplemental. (Green and Leung, 4/3/06).

Dog 8-Week Dietary Toxicity Study

0050, 218904; "BAJ 2740 Subchronic Toxicity Study in Male Dogs (8 Week Feeding Study)" (Wetzig, H. and Hartmann, E., Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report No. PH 30945, Bayer AG Study No. T 9 062 317, 04/19/01). BAJ 2740 (Mixed Batch No. 06480/0002, purity = 97.9-98.6%) was admixed to the diet, this admixture was then mixed with tap water at a 1:1 ratio, and this paste was then immediately offered to 5 male beagle dogs per dose at dose levels of 0 (diet only), 100, or 2000 ppm (0, 2.9, and 55.9 mg/kg/day, respectively) for 57 days. No mortalities occurred. No clinical signs were observed. No effect on body weight was observed. No effect on hematological parameters was observed. Treatment-related increases in aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, and alkaline phosphatase levels and a treatment-related decrease in cholesterol were observed at 2000 ppm. Treatment-related increases in activity of the liver enzymes 7-ethoxycoumarin deethylase and aldrin epoxidase at 2000 ppm were observed. A treatment-related increase in the mean luteinizing hormone level was observed at 2000 ppm. Treatment-related increases in mean relative adrenal and liver weights were observed at 2000 ppm. Microscopic examination revealed treatment-related zona fasciculata vacuolation of adrenal tissue at 100 and 2000 ppm, mononuclear cell infiltration in adrenal tissue at 2000 ppm, single cell necroses and more granular cytoplasm in the liver at 2000 ppm, slight hypertrophy and slight to moderate vacuolation of the Leydig cells in the testes at 2000 ppm, and multifocal degeneration of germinal epithelium of the testes at 2000 ppm. **Possible adverse effect indicated:** Leydig cell vacuolation in the testes. NOEL (M) could not be determined.

Supplemental study (only male animals were used, only 2 dose levels were used, and the test animals were treated for only 57 days). (Corlett, 01/10/06)

Dog Subchronic Dietary Toxicity Study

****52944-0031, 032 218873 and 218874**, "BAJ 2740, Subchronic Toxicity Study in Beagle Dogs (14 Week Feeding Study)", (H. Wetzig and E. Hartmann, Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Report Nos. PH 30661 and PH 30661A, Bayer AG Study No. T 4 061 566, Bayer Inc., Report No. 110504, 11 December 2000 and Report No. 110504-1, 12 March 2002). Record 218873 contained the study with results; 218874 contained historical control data for hematology, clinical chemistry, liver exams, urinalysis, and histopathology in Beagle dogs. 4 Beagle dogs per sex per group received BAJ 2740 (98.6% spirodiclofen) in the diet (moistened 1:1 with water) at 0 (standard diet), 200, 630, and 2000 ppm for 14 weeks. Mean BAJ 2740 intake (m + f) was 8.0, 27.3, and 82.8 mg/kg/day at 200, 630, and 2000 ppm respectively during the 14 week treatment period. At 630 and 2000 ppm, bodyweights were decreased (not significant) for males and females (from week 4) compared to controls during treatment. Erythrocytes, hemoglobin, and hematocrit values in both sexes were reduced relative to controls (but within the historical control range) at 630 and 2000 ppm from

week 6. Treatment-related clinical chemistry changes were noted at 630 and 2000 ppm. Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (Aph), and glutamate dehydrogenase (GLDH) activities were increased for mid-dose females at week 6 and for high dose males and females at weeks 6 and 13 relative to controls. Cholesterol and thyroxine (T4) values were decreased at 630 and 2000 ppm in both sexes, although values were within historical control parameters. Albumin values were decreased at 2000 ppm but fell within the historical control range. At 630 and 2000 ppm, protein levels fell below study control values at weeks 6 and 13 but were comparable to pretreatment levels. At necropsy, dose dependent increases in N- and O-demethylase activities were recorded at 200 ppm and higher. At 630 and 2000 ppm, slight increases in cytochrome P-450 were measured. Additionally, dose-dependent increases in the cytochrome P-450 dependent monooxygenases 7-ethoxycoumarin deethylase (ECOD) and aldrin epoxidase (ALD) were measured. Induction at 200 ppm was marginal, while at 630 and 2000 ppm, increases of 1.7 (females) to 2 (males) fold in ALD, and 3.2 (females) to 4.5 (males) fold in ECOD were noted compared to controls. Epoxide hydrolase (EH) was also induced in high dose females. Relative liver weights were increased for males and females at 630 and 2000 ppm. Dose-dependent increases in relative kidney, adrenal, and pituitary weights were noted for both sexes at 200 ppm and higher. Relative prostate weights were decreased for males at 200 ppm and higher. Histopathology revealed cytoplasmic change, inflammatory periportal infiltrates, and slight periportal single cell necroses in the livers of high dose females. Dilation of the proximal renal tubules was noted in both sexes at 2000 ppm. Vacuolation and hypertrophy/activation of Leydig cells in testes, degeneration and/or immaturity of the testicular germinal epithelium, oligospermia and/or aspermia of the epididymides, and prostatic immaturity were noted for mid and high dose males. Cytoplasmic vacuolation of the adrenal cortex was noted in mid and high dose males and at 200 ppm and above in females. Cortical vacuolation was combined, in many cases, with infiltration of mononuclear cells in dogs at all treatment levels. Additionally, a higher incidence of mild atrophy of the thymic cortex was noted in high dose animals. NOEL < 200 ppm (8.0 mg/kg/day) (based on increased incidence of vacuolation in the adrenal cortex). Possible adverse effect: vacuolation of the adrenal cortex. Acceptable. (Green and Leung, 4/5/06).

Rat 4-Week Repeated Dosing Dermal Toxicity Study

0032; 218875; "BAJ 2740, Study for Subacute Dermal Toxicity in Rats (Four-Week Treatment Period)" (Krötlinger, F. and Sander, E., Bayer AG, Department of Toxicology, Wuppertal, Germany, Bayer AG Study No. T2062338, Bayer AG Report No. 28712, 05/04/99). 822. BAJ 2740 (Mixed batch no. 06480/0002, purity = 97.9%) was moistened with water and applied to the shaved dorsal skin of 5 Wistar (HsdCpb:WU) rats per sex per dose at dose levels of 0 (tap water) or 1000 mg/kg/day for 6 hours per day 5 days per week for 4 weeks (7 days per week during the 4th week). No mortalities occurred. No clinical signs and no skin effects at the test site were observed. No toxicologically significant effect on body weight was observed. Hematological and clinical chemistry investigations revealed no toxicologically significant effects. No effects on organ weights were observed. Necropsies and microscopic examinations revealed no treatment-related effects. **No adverse effects.** NOEL (M/F, systemic and skin) = 1000 mg/kg/day (based on no effects at the highest dose tested). **Acceptable.** (Corlett, 01/25/06)
